Modulation of Intercellular Adhesion Molecule-1 Expression on Human Melanocytes and Melanoma Cells: Evidence for a Regulatory Role of IL-6, IL-7, TNFβ, and UVB Light

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Intercellular adhesion molecule-1 (ICAM-1) is involved in cell-cell interactions of leukocytes and parenchymal cells and thus plays an important role in immunologic and inflammatory reactions. The expression of ICAM-1 that is found on many different cells such as melanocytes and melanoma cells is induced by various cytokines, including interferon-gamma (IFNγ), interleukin (IL)-1 and tumor necrosis factor alpha (TNFα). Because expression of ICAM-1 in melanoma was found to correlate with increased risk of metastasis, the regulation of ICAM-1 expression on human melanocytes and melanoma cells was investigated. Foreskin-derived melanocytes and melanoma cell lines (A375, G361) were incubated with different cytokines and ICAM-1 expression was evaluated by fluorescence-activated cell sorter. IFNγ, IL-1, IL-7, TNFα, and TNFβ significantly upregulated ICAM-1 expression in a dose-dependent manner. Most interestingly, the cytokine IL-6, which does not influence adhesion-molecule expression on other cells, significantly upregulated melanocyte and melanoma cell ICAM-1 expression. This effect was dose dependent and could be blocked by an IL-6 antibody. Irradiation with ultraviolet (UVB) light did not influence constitutive ICAM-1 expression on melanoma cells and melanocytes, but suppressed cytokine-induced ICAM-1 expression when cells were harvested 16 h after irradiation. These findings were further confirmed by Northern blot analysis, showing a marked accumulation of ICAM-1 mRNA after cytokine treatment, which was reduced by irradiation with UVB light. However, when UVB-exposed melanoma cells were cultured for at least 48 h induction of ICAM-1 expression was observed. These data indicate that, similar to other cells, ICAM-1 expression on melanoma cells and melanocytes is regulated by cytokines and that UVB light affects ICAM-1 expression on melanocytic cells in a biphasic manner. *J Invest Dermatol 98:320–326, 1992*

The leukocyte integrins (Leu-CAM) comprise a family of three glycoproteins termed LFA-1, Mac-1, and p150,95. In contrast to the other members of the integrin family, which act as receptors for extracellular matrix components, the Leu-CAM are involved in intercellular adhesion reactions [1,2]. Adhesion via LFA-1, which is expressed by all leukocytes, appears to be crucial in the development of most lymphocyte-mediated responses, including helper T-lymphocyte responses, cytotoxic T-cell reactions, and adhesion of lymphocytes to endothelial cells and fibroblasts. The adhesive ligand for LFA-1 is intercellular adhesion molecule-1 (ICAM-1, CD54), a cell-surface glycoprotein that belongs to the immunoglobulin superfamily [3–5]. ICAM-1 is found on a variety of cells including activated mononuclear leukocytes, fibroblasts, vascular, endothelial, and epithelial cells. Its expression is induced or increased within hours by inflammatory mediators, such as interferon-gamma (IFNγ), interleukin (IL)-1, and tumor necrosis factor-alpha (TNFα) [5–7]. There is strong evidence for an integral role of LFA-1/ICAM-1 interaction in antigen-specific responses because it has been demonstrated that monoclonal antibodies to either LFA-1 or ICAM-1 block the allogenic cytotoxic T-lymphocyte–mediated killing and proliferation of T cells after antigenic stimulation [8,9].

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Abbreviations.

bFGF: basic fibroblast growth factor
FACS: fluorescence-activated cell sorter
GM-CSF: granulocyte/macrophage colony-stimulating factor
ICAM-1: intercellular adhesion molecule-1
IFNγ: interferon-gamma
IL: interleukin
LAK: lymphokine-activated killer cells
LFA: lymphocyte function–associated antigen
MEM: modified Eagle's medium
MHC: major histocompatibility complex
MoAb: monoclonal antibody
rh: recombinant human
TGFβ: transforming growth factor-beta
TNF: tumor necrosis factor
UV: ultraviolet

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In addition, antigen-independent responses mediated by ICAM-1/LFA-1 interaction have been demonstrated for natural killer cells and lymphokine-activated killer (LAK) cell cytotoxicity [10,11].

Human keratinocytes are known to express ICAM-1, which can be upregulated by stimulation with cytokines like IFNγ and TNFα, but not with IL-1 or IL-6 [12,13]. Although ICAM-1 is not found on keratinocytes in normal human skin in situ [14,15], under pathologic conditions, e.g., psoriasis or patch test reaction keratinocytes express remarkable amounts of ICAM-1, suggesting that ICAM-1 may be of relevance in inflammatory and immune responses in the skin, e.g., by facilitating lymphocytic infiltration.

Recently, it was shown that normal human melanocytes also express ICAM-1 [16]. In addition, a 96-kD melanoma-associated antigen was cloned and found to be identical to ICAM-1 [17,18]. Currently, it has been reported that the expression of ICAM-1 in melanoma correlates with increased risk of metastasis [19]. Therefore the regulation of ICAM-1 on melanocytic cells appears to be of interest. Accordingly, it has been described that the cytokines IL-1, TNFα, and IFNγ upregulate ICAM-1 on melanocytes [16]. Within the last few years, it has turned out that the epidermis and in particular the keratinocyte is a potent source for a variety of cytokines including IL-1, IL-6, IL-8, colony-stimulating factors, TNF, and growth factors [20]. In order to obtain more insight into the regulation of ICAM-1 expression on melanocytic cells, the effects of the epidermal cell-derived cytokines IL-6 [21] and IL-7 [22], and of TNFβ, another well known inducer of ICAM-1 on keratinocytes [13], were studied. In addition, the influence of ultraviolet (UV) light on the ICAM-1 expression of melanocytic cells was investigated, because melanocytes and melanoma cells by virtue of their anatomic location are exposed to UV light, which is well known for its inflammatory and immunoregulatory capacities.

MATERIALS AND METHODS

Cell Culture Primary cultures of human melanocytes were established from neonatal foreskin obtained from five individuals. Foreskin samples were incubated for 16 h at 4°C in 0.25% trypsin/0.02% EDTA in PBS without Ca^{2+} and Mg^{2+}. Subsequently, the epidermis was separated from the dermis, washed in PBS containing 10% FCS, and recovered by centrifugation. The cell pellet was resuspended in melanocyte growth medium and incubated at 37°C in humidified atmosphere containing 5% CO_2. Melanocyte growth medium consisted of Ham's F10 medium (Flow, Irvine, Scotland) supplemented with 10% Nu-serum, bovine pituitary extract (5 mg/100 ml, CR, Bedford, MA), 2% FCS, penicillin/fungizone (Gibco, Paisley, Scotland), 12-O-tetradecanoylphorbol 13-acetate (TPA, 4.8×10^{-8} M), isobutyl methylxanthine (IBMX, 1×10^{-4} M), and cholera toxin (2.5×10^{-9} M) (Sigma Chemical, St. Louis, MO). Confluent cultures were trypsinized and passaged 1:3 into 6-well plates for fluorescence-activated cell sorter (FACS) analysis or culture petri dishes (15 cm diameter, Falcon, Osnard, CA) for Northern blot analysis and cultured in defined serum-free medium at least 7 d prior to ICAM-1 analysis.

The human melanoma cell lines A375 and G361 (ATCC, Rockville, MD) were maintained in Dulbecco's modified Eagle's medium (MEM) (Gibco, Paisley, Scotland) supplemented with 10% heat-inactivated FCS, 2 mM glutamin, and penicillin/streptomycin.

Cytokines and Antibodies Recombinant human (rh) IL-2 and rhIL-6 were purchased from Collaborative Research (Bedford, MA), rhTNFβ and rhIL-7 were from British Biotechnology (Oxford, UK), rhIL-8 were obtained from Biosource (Westlake Village, CA), rhIFNγ were from Bender (Vienna, Austria), rhTNFα was purchased from Endogen (Boston, MA), rhIL-1α, rhIL-1β, rhGM-CSF and mouse monoclonal antibodies directed against rhIL-6, and rhTNFβ were obtained from Boehringer (Mannheim, FRG). A monoclonal anti-ICAM-1 antibody (IgG1) was obtained from Immunotech (Marseille, France), a monoclonal anti-CD4 antibody (IgG1, Becton & Dickinson, Mountain View, CA) was used as an isotype control. FITC staining was performed with a FITC-conjugated goat-anti-mouse $F(ab')_2$ antibody (Grub, Kaumberg, Austria).

Irradiation with Ultraviolet Light Medium of subconfluent cultures was replaced by pre-warmed PBS (2 ml for a 6-well culture plate of 20 ml for a petri dish) and irradiated with doses of UVB-light ranging from 25 to 125 J/m^2 using a bank of 4 FS 20 bulbs (Westinghouse Electric Corp., Pittsburg, PA), which emit most of their energy within the UVB range (290-320 nm) with a peak at 313 nm as described previously [23]. Immediately after phototreatment cells were washed and cultured in serum-free medium in the absence or presence of various cytokines. Cell viability was determined by trypan blue exclusion and did not significantly differ from unirradiated control cells.

ICAM-1 Detection by FACS Analysis After an incubation of 16 h (unless otherwise indicated) in 6-well culture dishes cells were detected with 0.02% EDTA (melanocytes) or trypsinized (melanoma cells), incubated with anti-ICAM monoclonal antibody (MoAb) (1:40) or anti-CD4 (1:40) as an isotype control for 30 min at 4°C, washed, and incubated with a 1:20 diluted FITC-conjugated goat-anti-mouse $F(ab')_2$ secondary antibody for 30 min at 4°C in the dark. Expression of ICAM-1 was analyzed using a FACS SCAN (Becton & Dickinson, Mountain View, CA). Data are given either as histograms (y-axis indicating number of cells, x-axis fluorescence intensity) or as percentage of ICAM-1-positive cells. The expression was determined as the difference between cells stained with anti-ICAM-1 and cells stained with the isotype-matched irrelevant (anti-CD4) control antibody. Each experiment was performed at least three times; figures show the data of one representative experiment.

Northern Blot Analysis Total cellular RNA was isolated by guanidinium isothiocyanate-cesium chloride ultracentrifugation, electrophoresed on 1% agarose/2.2 M formaldehyde gels, and vacuum blotted onto nylon membranes (Bio-Rad, Richmond, CA). Equivalent loading, transfer, and integrity of the RNA was monitored by ethidium bromide staining or hybridization with a housekeeping gene (β-actin). Pre-hybridization was carried out for 4 to 6 h at 42°C in 40% formamide, 4 × SSC, 1 × Denhardt's solution, 10 mM Tris (pH 4.7), 50 mg/ml sheared and denatured salmon sperm DNA, and 50 mg/ml yeast tRNA. Hybridization was carried out using a ^{32}P-labeled 3-kb Sal/EcoRI ICAM-1 cDNA probe (kindly provided by T.A. Springer, Boston) or a ^{32}P-labeled 3.6-kb HindIII human β-actin cDNA (kindly provided by D. Gallwitz, Göttingen, FRG) as a control. After overnight hybridization, membranes were washed under high-stringency conditions (0.1 × SSC, 65°C) and exposed to XAR-5 films (Kodak Corp., Rochester, NY) using intensifying screens at −70°C for 7 d.

RESULTS

rhIL-6, rhIL-7, or rhTNFβ Treatment Induce ICAM-1 Cell Surface Expression on Human Melanocytes and Melanoma Cell Lines When unstimulated human foreskin melanocytes were stained with ICAM-1 antibody, no or only minimal amounts of ICAM-1 surface expression could be detected (Fig 1). However, incubation with 500 U/ml IFN for 16 h led to a significant induction of ICAM-1 on melanocytes (Fig 1). A similar upregulation was observed upon treatment with rhIL-6 (1 μg/ml), rhIL-7 (0.1 μg/ml), or rhTNFβ (5000 U/ml). In contrast, the human melanoma cell lines G361 (Fig 1) and A375 (data not shown) expressed significant amounts of ICAM-1 on their cell surface constitutively, which were induced by rhIL-6, rhIL-7, or rhTNFβ (Fig 2). Normal melanocytes responded identically to the same treatment with a marked upregulation of ICAM-1 (Fig 2).

Whereas the inducing effect of TNFβ is in accordance with recent findings on keratinocytes [13], IL-6 so far has failed to affect ICAM-1 expression on various cell species including keratinocytes and endothelial cells [13,24]. In order to rule out that this new biologic activity of IL-6 was due to endotoxin contamination of rhIL-6, antibody-blocking experiments were performed. Pre-incubation of rhIL-6 with an anti-IL-6 antibody but not with an irrelevant control antibody specifically neutralized the inducing effect of IL-6 on melanoma ICAM-1 expression (Fig 3). In addition, the ICAM-1-inducing effect on melanoma cells by IL-6 was dose de-

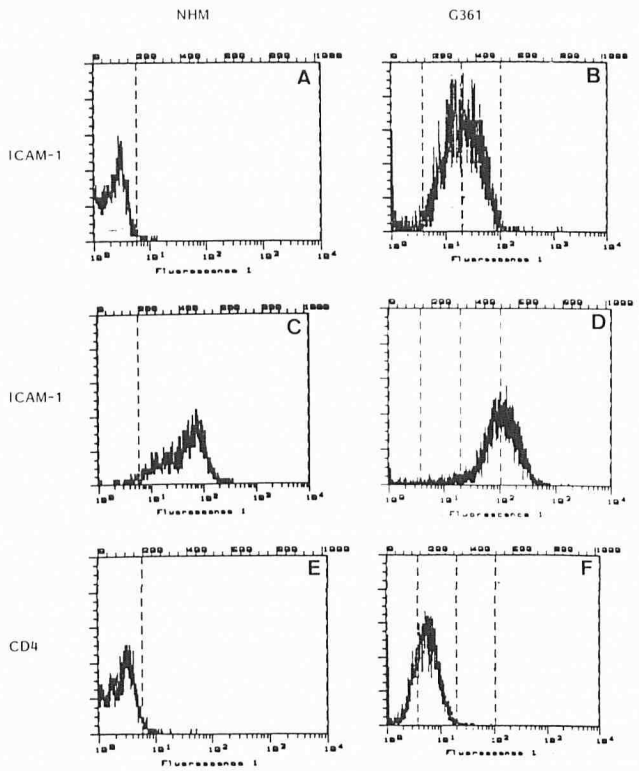

Figure 1. ICAM-1 expression on normal human melanocytes (NHM) and G361 melanoma cells. NHM and G361 cells were left untreated *(A,B)* or stimulated with IFNγ (500 U/ml) for 16 h *(C,D)* and evaluated for ICAM-1 expression by FACS analysis. An anti-CD4 antibody was used as an isotype control *(E,F)*.

pendent (the percentage increase of ICAM-1 expression on G361 cells was, for 1 μg/ml, 28.8%; for 100 ng/ml, 21.2%; for 1 ng/ml, 13.4%; and for 0.1 ng/ml, 0%).

Because melanocytes and melanoma cells appeared to be the first cells responding to IL-6 with upregulation of ICAM-1, a panel of other cytokines was tested. As demonstrated in Table I, IL-2, IL-8, granulocyte/macrophage colony-stimulating factor (GM-CSF), transforming growth factor beta (TGFβ), and basic fibroblast growth factor (bFGF) within a wide dose range did not affect ICAM-1 on human melanoma cell lines, whereas IFNγ, IL-1α, IL-1β, and TNFα, well known inducers on other cells, identically increased ICAM-1 levels on melanoma cells (Table I).

UVB Irradiation Suppresses Cytokine-Induced ICAM-1 Expression UVB irradiation has been shown to influence the production of cytokines and to differentially affect ICAM-1 on keratinocytes [13,25,26]. Therefore, unstimulated A375 cells were irradiated with 100 J/m^2 UVB light, which did not affect ICAM-1 expression as analyzed after 16 h (Fig 4*A*). However, if cells were irradiated with UVB light followed by an immediate stimulation with inducing cytokines (IL-6, TNFα, TNFβ, or IFNγ) increase in ICAM-1 expression was no longer observed (Fig 4*B,C,D*). This inhibitory effect of UVB light on cytokine-induced ICAM-1 expression was dose dependent; 125 J/m^2 UVB caused maximum unresponsiveness to cytokines both in normal melanocytes and melanoma cell lines (data not shown). These data indicate that UVB light does not affect constitutive ICAM-1 expression, but downregulates ICAM-induction by cytokines, if cells are evaluated by FACS analysis within 16 h after treatment. However, when A375 cells exposed to UVB in the absence of cytokines were cultured beyond 16 h an upregulation of ICAM-1 expression was observed; maximal levels were detected on cells harvested 72 h after UV exposure, suggesting a biphasic response of melanoma cells to UV light as recently suggested for keratinocytes (Fig 5). Similar results were obtained with G361 cells (data not shown).

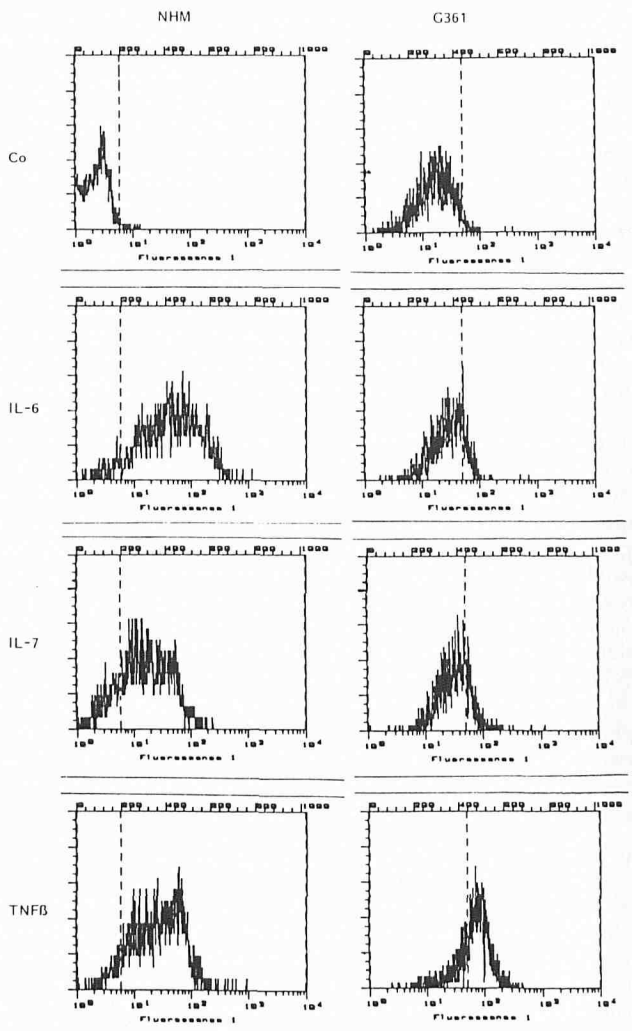

Figure 2. Induction of ICAM-1 expression on normal human melanocytes (NHM) and G361 melanoma cells. NHM and G361 cells were incubated with rhIL-6 (1 μg/ml), rhIL-7 (0.1 μg/ml), or TNFβ (5000 U/ml) or left untreated for 16 h and evaluated for ICAM-1 expression FACS analysis.

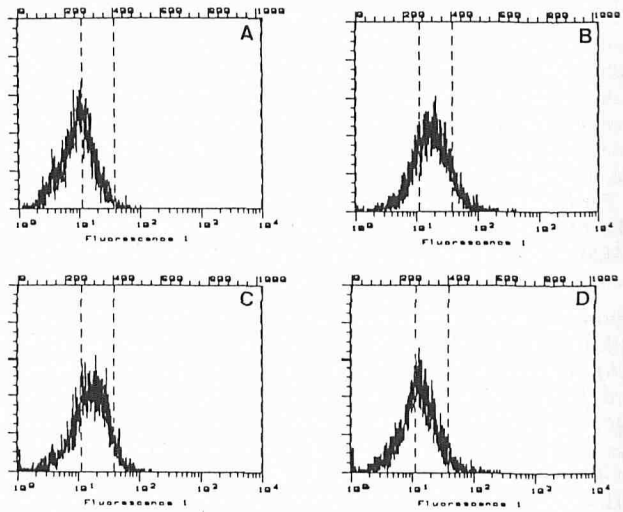

Figure 3. Induction of ICAM-1 on G361 melanoma cells by rhIL-6. G361 cells were treated as follows: no treatment *(A)*; rhIL-6 (1 μg/ml) *(B)*; rh IL-6 in the presence of an antibody directed against IL-1β (isotype control) *(C)*; or rh IL-6 in the presence of an IL-6 antibody *(D)*. After incubation for 16 h cells were evaluated for ICAM-1 expression by FACS analysis.

Table I. Effect of Cytokines on ICAM-1 Expression by Melanoma Cells

Cytokine	Concentrations Tested (Specific Activity)	Maximum Induction	Increase ICAM-1[a] Positive Cells (%)
rhIL-1α	0.1–100 U/ml (1×10^8 U/mg)	100 U/ml	22.9
rhIL-1β	1–100 U/ml (5×10^8 U/mg)	100 U/ml	23.4
rhIL-2	10–1,000 U/ml (5×10^6 U/mg)		0.2
rhIL-6	1 pg–1 μg/ml	1 μg/ml	28.8
rhIL-7	1 ng–1 μg/ml	1 μg/ml	18.2
rhIL-8	1 ng–1 μg/ml		0.7
rhGM-CSF	1–100 U/ml ($1.25–5 \times 10^7$ U/mg)		0.6
rhTNFα	0.05–50 U/ml (2×10^6 U/mg)	50 U/ml	61.0
rhTNFβ	50–5,000 U/ml (2×10^6 U/mg)	5,000 U/ml	85.1
rhIFNγ	5–50,000 U/ml (2.5×10^7 U/mg)	50,000 U/ml	95.2
rhTGFβ	0.1–10 ng/ml ($1–2 \times 10^7$ U/mg)		0.5
rhbFGF	5–20 ng/ml ($1–5 \times 10^7$ U/mg)		0.2

[a] G361 melanoma cells were incubated with the respective cytokines in the concentrations indicated and ICAM-1 expression evaluated by FACS analysis after an incubation of 16 h. Data are expressed as percent increase in comparison to untreated control cells.

Cytokine Treatment and UVB Irradiation Transcriptionally Regulate ICAM-1 Expression in Human Melanocytes and Melanoma Cell Lines To study the mechanism involved in the regulation of ICAM-1 expression by pigment cells, Northern blot analysis was performed. Melanocytes were incubated for 4 h with 1 μg/ml IL-6 or left untreated. Subsequently, cytoplasmic RNA was extracted and hybridized with an ICAM-1 cDNA probe. Unstimulated melanocytes did not express detectable ICAM-1 mRNA (Fig 6, *lane 1*), which, however, was inducible by IL-6 (Fig 6, *lane 3*). In G361 melanoma cells ICAM-1–specific RNA was found constitutively expressed and significantly upregulated by treatment of cells with IL-6 (Fig 7, *lane 2*) or IL-7 (Fig 8, *lane 2*), suggesting that these cytokines, similar to IFNγ and TNFα, affect ICAM-1 expression at the level of transcription.

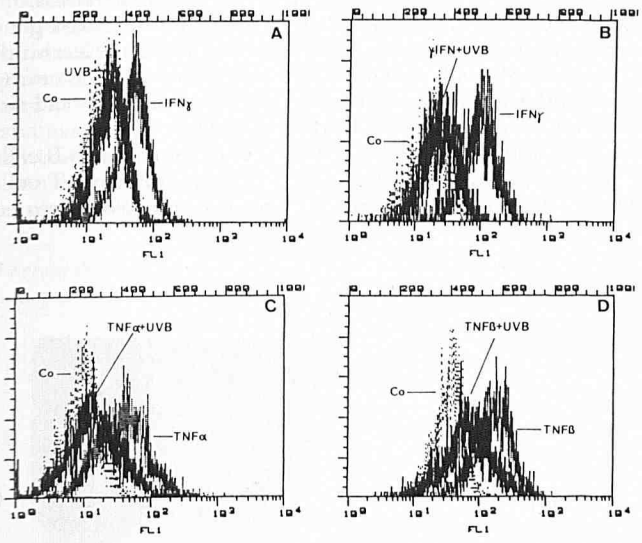

Figure 4. Effect of UVB irradiation on constitutive and cytokine-induced ICAM-1 expression. *A*: A375 cells were irradiated with UVB light (100 J/m²) and ICAM-1 expression compared to untreated (Co) or IFNγ (500 U/ml) treated cells. *B*: A375 cells were irradiated with UVB light and immediately after exposure treated with IFNγ (IFNγ + UVB) and ICAM-1 expression compared to untreated (Co) or IFNγ-treated (IFNγ) cells. *C*: A375 cells were irradiated with UVB light and immediately after exposure treated with TNFα (50 U/ml; TNFα + UVB) and ICAM-1 expression compared to untreated (Co) or TNFα-treated (TNFα) cells. *D*: A375 cells were irradiated with UVB light and immediately after exposure treated with TNFβ (5000 U/ml); TNFβ + UVB) and ICAM-1 expression compared to untreated (Co) or TNFβ-treated (TNFβ) cells. Cells were incubated for 16 h and evaluated for ICAM-1 expression by FACS analysis.

Exposure of melanocytes to UVB alone did not affect ICAM-1 mRNA isolated 4 h after irradiation (Fig 6, *lane 2*) when compared to untreated controls (Fig 6, *lane 1*). However, treatment with UVB light immediately before stimulation with rhIL-6 for 4 h reduced IL-6–induced ICAM-1 mRNA upregulation in human melanocytes (Fig 6, *lane 4*). Similarly, UVB suppressed the induction of ICAM-1 mRNA in melanoma cells, (Fig 8, *lane 1*). These results suggest that UVB might interfere reversibly with cytokine receptor–mediated signal transduction, which prevents transcriptional activation of the ICAM-1 gene. Similar observations have been made in human keratinocytes and carcinoma cell lines [13].

In order to study the mechanisms involved in ICAM-1 upregulation by UVB light in the late phase, A375 melanoma cells were irradiated with UVB and cultured for 48 h, and RNA extracted. UVB irradiation resulted in a significant increase of ICAM-1–specific mRNA (Fig 9, lane 2).

DISCUSSION

Because there is recent evidence for a correlation between ICAM-1 expression on melanoma cells and tendency to metastasize [19], the regulation of ICAM-1 seems to be an important issue. Currently, it was shown that normal human melanocytes express ICAM-1 and that this is influenced by the cytokines IL-1, TNFα, and IFNγ [16]. Because the epidermis is a source for a variety of mediators [20] the effect of other keratinocyte-derived cytokines was studied.

In our hands, human foreskin–derived melanocytes in early passages were found to lack constitutive expression of ICAM-1. This is in contrast with recent reports [16,17]. The reason for this differ-

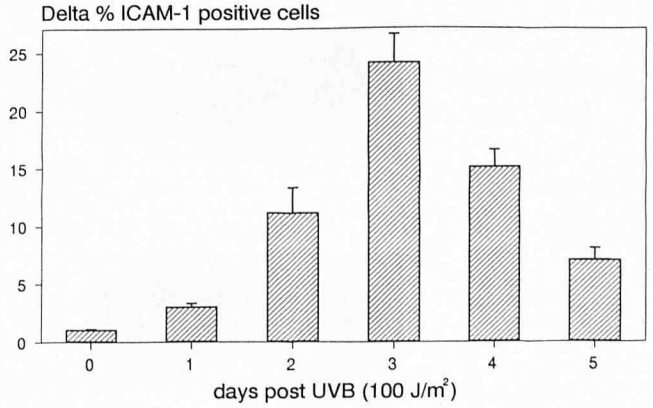

Figure 5. Time kinetics of ICAM-1 expression on A375 cells after UVB exposure. A375 cells were irradiated with UVB light (100 J/m²) and ICAM-1 expression evaluated by FACS analysis after 24, 48, 72, 96, or 120 h.

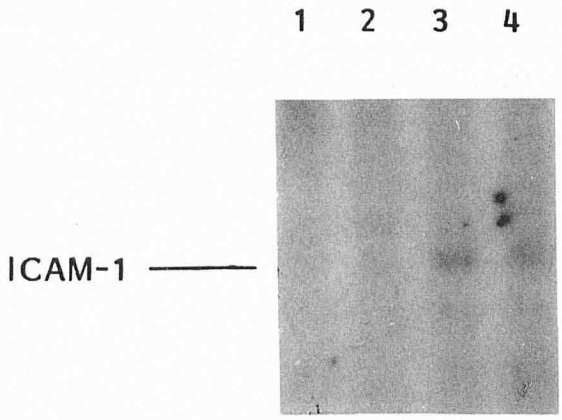

Figure 6. Modulation of ICAM-1 expression in melanocytes by IL-6 and UVB. Melanocytes were treated as follows: *Lane 1,* no treatment; *lane 2,* UVB (100 J/m²); *lane 3,* IL-6 (1 μg/ml); *lane 4,* UVB (100 J/m²) plus IL-6. RNA was extracted 4 h after stimulation and hybridized with a cDNA probe encoding for ICAM-1.

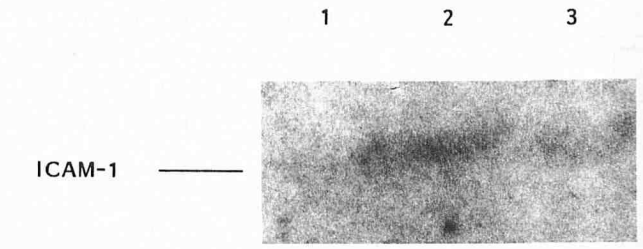

Figure 8. Modulation of ICAM-1 expression in G361 cells by IL-7 and UVB. G361 cells were irradiated with UVB light (100 J/m²) followed by IL-7 stimulation (1) or stimulated with IL-7 (0.1 μg/ml) alone (2) or left untreated (3). RNA was extracted 4 h after stimulation and hybridized with a cDNA probe encoding for ICAM-1.

ence may be due to different culture conditions; however, stimulation of melanocytes with IFNγ, TNFα, or IL-1 for 16 h strongly induced ICAM-1, which is in accordance with previously reported results [16].

Keratinocytes are known to release a variety of mediators [20] including TNFα and IL-1 that influence ICAM-1 expression. Recently, keratinocytes have been shown to be capable of producing the multifunctional cytokine IL-6 [21,27], which appears as a mediator of pathologic processes within the skin. Accordingly, increased IL-6 production has been found after UV exposure [23,28] and in psoriasis [29,30]. Therefore, it had been obvious to test whether IL-6 affects ICAM-1 expression. However, in the cell species so far studied including keratinocytes and endothelial cells IL-6 had no effect on ICAM-1 regulation [13,24]. The present study demonstrates for the first time that IL-6 is able to induce ICAM-1 on melanocytic cells, thus revealing a new biologic property of this cytokine. The ICAM-1–inducing effect of IL-6 is dose dependent and still detectable in the ng/ml range. Whether this concentration is sufficient to function in vivo cannot be answered yet, because to the best of our knowledge reliable tissue concentrations within the epidermis have not been determined. Melanocytes, however, appear to be more sensitive than keratinocytes, because much lower doses of IFNγ were necessary to modulate ICAM-1 on melanocytes [16] and IL-1 affects ICAM-1 expression on melanocytes, whereas keratinocytes are resistant to IL-1 [12,16].

The possibility that the induction of ICAM-1 by IL-6 was due to lipopolysaccharide contamination was ruled out by specifically blocking the effect with an IL-6 antibody. The mechanism by which IL-6 selectively enhances ICAM-1 expression in melanocytic cells but not in keratinocytes is known. Both keratinocytes [31] and melanoma cells (unpublished observations) seem to express IL-6 receptors. Whether there exist differences in IL-6 receptor structure and function and/or signal transduction mechanisms that could regulate ICAM-1 expression in a cell- and tissue-specific manner remains to be determined.

To analyze whether the expression of ICAM-1 is transcriptionally regulated by IL-6, IL-7, and TNFβ as described for other cytokines [13,32], Northern blot analysis was performed, which showed clearly inducible ICAM-1 message by IL-6 in melanocytes. However, our data do not exclude additional post-transcriptional control mechanisms such as stabilization of mRNA or increased translation. The recent cloning of the 5′-regulatory region of the ICAM-1 gene and identification of consensus binding sites for several nuclear binding proteins [33] should enable the detailed analysis of the cis-acting elements and trans-activating proteins responsible for cell- and tissue-specific control of ICAM-1 expression [34].

IL-7 was originally found to induce proliferation of pre–B cells [35]. Recently, it was observed that IL-7 induces cytotoxic T cells and LAK cells [36,37]. Moreover, IL-7 induces de novo or increased

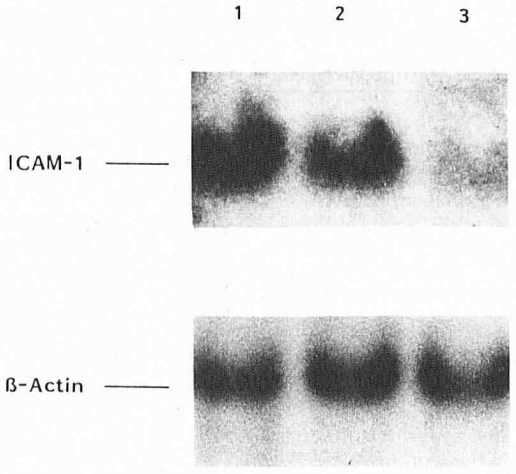

Figure 7. Modification of ICAM-1 expression on G361 cells by IL-6 or IFNγ. G361 cells were stimulated with IFNγ (500 U/ml) (1) or IL-6 (1 μg/ml) (2) or left untreated (3). RNA was extracted 4 h after stimulation and hybridized with a cDNA probe encoding for ICAM-1.

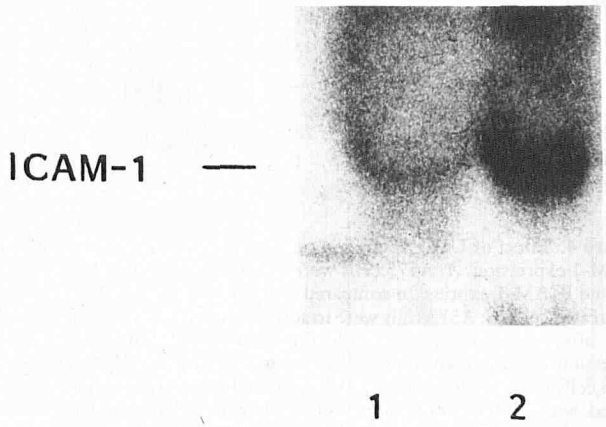

Figure 9. Modulation of ICAM-1 expression in A375 cells by UVB light. A375 cells were left untreated (1) or irradiated with UVB light (100 J/m²) (2) and cultured for 48 h. RNA was extracted after 48 h and hybridized with a cDNA probe encoding for ICAM-1.

expression of the IL-2 receptor β-chain, CD69, and ICAM-1 on LAK cells [38]. The present study confirms the upregulating effect of IL-7 on ICAM-1 and shows that this activity is not confined to leukocytes. This observation appears to be of relevance because recently murine keratinocyte cell lines were found to release IL-7 [22]. In addition, this study provides evidence that rhTNFβ upregulates ICAM-1 expression on melanocytes and melanoma cells. This is in accordance with recent reports showing that TNFβ is able to induce ICAM-1 on human fetal astrocytes, endothelial cells, and keratinocytes [13,38,39]. Because TNFβ is primarily a T-cell-derived product, this may be of relevance for the expression of ICAM-1 in melanocytic tumors surrounded by infiltrating T lymphocytes.

The importance of the upregulation of cell-surface molecules such as HLA-DR, ICAM-1, and lymphocyte function-associated antigen-3 as seen on different melanomas and normal melanocytes by various cytokines is demonstrated by the recent findings that these molecules are particularly important for effective antigen presentation [40] in MHC-restricted as well as MHC-non-restricted lymphocyte responses. Therefore cytokines released within inflammatory reactions may induce ICAM-1 expression on melanocytes and render them susceptible as targets for cytotoxic T cells and natural killer cells, thus causing post-inflammatory hypopigmentation [16].

The biologic effects of UVB light are multiple and include suppression of the immune response [41], which contributes to carcinogenesis and may participate in the generation of malignant melanoma [42,43]. The effects of UV light on ICAM-1 expression on both melanocytes and melanoma cells appear to be biphasic, producing inhibition of cytokine-induced ICAM-1 expression within the first 16 h and transient induction at 48, 72, and 96 h. These observations are similar to those found with keratinocytes [26]. Whether the induction in the late phase is due to an autocrine stimulation of cytokines released by melanocytic cells remains to be determined. Because the secretion of TNFα by keratinocytes is induced by UV light [44], keratinocytes may contribute to ICAM-1 regulation on melanocytic cells in a paracrine manner.

Therefore, the influence of UVB irradiation in vivo on ICAM-1 expression by pigment cells is likely to be the sum of direct and indirect, i.e., cytokine-mediated effects. However, the exact biochemical mechanisms by which UVB perturbs ICAM-1 expression remains to be determined. In addition, ICAM-1 expression may not only drive migration of leukocytes to the skin, but may also occur as a consequence of local accumulation of mediators released by lymphoid cells infiltrating the skin or tumors.

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REFERENCES

1. Patarroyo M, Makgoba MW: Leucocyte adhesion to cells in immune and inflammatory responses. Lancet II: 1139-1142, 1989
2. Springer TA: Adhesion receptors of the immune system. Nature 346:425-433, 1990
3. Rothlein R, Dustin L, Marlin SD, Springer TA: A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. J Immunol 137:1270-1274, 1986
4. Marlin SD, Springer TA: Intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen-1 (LFA-1). Cell 51:831-843, 1987
5. Dustin ML, Staunton DE, Springer TA: Supergene families meet in the immune system. Immunol Today 9:213-215, 1988
6. Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA: Induction by IL-1 and interferon-G: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J Immunol 137:245-254, 1986
7. Barker JNWN, Allen MH, MacDonald DM: The effect of in vivo interferon-gamma on the distribution of LFA-1 and ICAM-1 in normal human skin. J Invest Dermatol 93:439-442, 1989
8. Siu G, Hedrick SM, Brian AA: Isolation of the murine intercellular adhesion molecule 1 (ICAM-1) gene. ICAM-1 enhances antigen-specific T cell activation. J Immunol 143:3813-3820, 1989
9. Dang LH, Michalek MT, Takei F, Benaceraff B, Rock KL: Role of ICAM-1 in antigen presentation demonstrated by ICAM-1 defective mutants. J Immunol 144:4082-4091, 1990
10. Timonen T, Patarroyo M, Gahmberg CG: CD11a-c/CD18 and GP84 (LB-2) adhesion molecules on human large granular lymphocytes and their participation in natural killing. J Immunol 141:1041-1046, 1988
11. Maio M, Tessitori G, Pinto A, Temponi M, Colombatti A, Ferrone S: Differential role of distinct determinants of intercellular adhesion molecule-1 in immunologic phenomena. J Immunol 143:181-188, 1989
12. Dustin ML, Singer KH, Tuck DT, Springer TA: Adhesion of T-lymphoblasts to epidermal keratinocytes is regulated by interferon gamma and is mediated by intercellular adhesion molecule-1 (ICAM-1). J Exp Med 167:1323-1340, 1988
13. Krutmann J, Köck A, Schauer E, Parlow F, Möller A, Kapp A, Förster E, Schöpf E, Luger TA: Tumor necrosis factor-β and ultraviolet radiation are potent regulators of human keratinocyte ICAM-1 expression. J Invest Dermatol 95:127-131, 1990
14. Vejlsgaard GS, Ralfkiaer E, Avnstrop C, Czajkowski M, Marlin SD, Rothlein R: Kinetics and characterization of intercellular adhesion molecule-1 (ICAM-1) expression on keratinocytes in various inflammatory skin lesions and malignant cutaneous lymphomas. J Am Acad Dermatol 20:780-782, 1989
15. Singer KH, Tuck DT, Sampson HA, Hall RP: Epidermal keratinocytes express the adhesion molecule intercellular adhesion molecule-1 in inflammatory dermatosis. J Invest Dermatol 92:746-750, 1989
16. Yohn JJ, Critelli M, Lyons MB, Norris DA: Modulation of melanocyte intercellular adhesion molecule-1 by immune cytokines. J Invest Dermatol 90:233-237, 1990
17. Tsujisaki M, Igarashi K, Eisinger M, Herlyn M, Ferrone S: Immunochemical and functional analysis of HLA class II antigens by recombinant immune interferon on normal human melanocytes. J Immunol 138:1310-1316, 1987
18. Temponi M, Romano G, D'Urso CM, Wang Z, Kekish U, Ferrone S: Profile of intercellular adhesion molecule-1 (ICAM-1) synthesized by human melanoma cell lines. Semin Oncol 15:595-607, 1988
19. Johnson JP, Stade BG, Holzmann B, Schwäble W, Riethmüller BG: De novo expression of intercellular-adhesion molecule-1 in melanoma correlates with increased risk of metastasis. Proc Natl Acad Sci USA 86:641-644, 1989
20. Schwarz T, Luger TA: Pharmacology of cytokines in the skin. In: Mukhtar H (ed.). Pharmacology of the Skin. CRC Press, Boca Raton, FL (in press)
21. Kirnbauer R, Köck A, Schwarz T, et al: Interferon-β2, B-cell stimulatory factor-2, hybridoma growth factor (interleukin-6) is expressed and released by human epidermal cells and epidermoid carcinoma cell lines. J Immunol 142:1922-1928, 1989
22. Heufler C, Young D, Peschel G, Schuler G: Murine keratinocytes express interleukin-7 (abstr). J Invest Dermatol 94:534A, 1990
23. Kirnbauer R, Köck A, Neuner P, et al: Regulation of epidermal cell interleukin-6 production by UV light and corticosteroids. J Invest Dermatol 96:484-489, 1991
24. Detmar M, Tenorio S, Imcke E, Ruszczak Z, Orfanos CE: TNF-alpha but not IL-6 induces HLA-DR and ICAM-1 expression in cultured dermal microvascular endothelial cells. Anti-TNF AB and ZNSO4 inhibit this induction (abstr). J Invest Dermatol 94:519, 1990
25. Schwarz T, Luger TA: Effect of UV-irradiation on epidermal cytokine production. J Photochem Photobiol B4:1-13, 1989
26. Norris DA, Lyons B, Middleton MH, Yohn JJ, Kashihara-Sawami M: Ultraviolet radiation can either suppress or induce expression of intercellular adhesion molecule-1 (ICAM-1) on the surface of cultured human keratinocytes. J Invest Dermatol 95:132-138, 1990
27. Wong GG, Clark SC: Multiple actions of interleukin 6 within a cytokine network. Immunol Today 9:137-139, 1988
28. Urbanski A, Schwarz T, Neuner P, et al: Ultraviolet light induces increased circulating interleukin-6 in humans. J Invest Dermatol 94:808-811, 1990
29. Neuner P, Urbanski A, Trautinger F, et al: Increased IL-6 production

by monocytes and keratinocytes in patients with psoriasis. J Invest Dermatol 97:27–33, 1991

30. Grossman RM, Krueger J, Yourish F, et al: Interleukin-6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc Natl Acad Sci USA 86:6367–6371, 1989
31. Krueger JG, Krane JF, Carter DM, Gottlieb AB: Role of growth factors, cytokines and their receptors in the pathogenesis of psoriasis. J Invest Dermatol 94(suppl):135S–140S, 1990
32. Li LJ, Degitz K, Caughman SW: The differential effects of transforming growth factor β (TGF-β) on interferon-g-induced expression of intercellular adhesion molecule (ICAM)-1 and DRβ in human keratinocytes (abstr). J Invest Dermatol 94:549, 1990
33. Degitz K, Li LJ, Caughman SW: Cloning and characterization of the transcriptional regulatory region of the human intercellular adhesion molecule-1 (ICAM-1) gene (abstr). J Invest Dermatol 94:518, 1990
34. Degitz K, Taylor J, Cornelius L, Li LJ, Caughman SW: Characterization of the transcriptional regulatory region of the human ICAM-1 gene responsive to interferon-gamma (abstr). J Invest Dermatol 96:533, 1991
35. Namen AE, Schmierer AE, March CJ, et al: B-cell precursor growth-promoting activity: purification and characterization of a growth factor active on lymphocyte precursors. J Exp Med 167:988–992, 1988
36. Hickman CJ, Crim JA, Mostowski HS, Siegel JP: Regulation of human cytotoxic T lymphocyte development by IL-7. J Immunol 145:2415–2420, 1990
37. Stötter H, Custer MC, Bolton ES, Guedez L, Lotze MT: IL-7 induces human lymphokine-activated killer cell activity and is regulated by IL-4. J Immunol 146:150–155, 1991
38. Frohman EM, Frohman TC, Dustin ML, et al: The induction of intercellular adhesion molecule-1 (ICAM-1) expression on human fetal astrocytes by interferon-gamma, tumor necrosis factor alpha, lymphotoxin, and interleukin-1: relevance to intracerebral antigen presentation. J Neuroimmunol 23:117–124, 1989
39. Pober JS, Lapierre LA, Stolpen AH, et al: Activation of cultured human endothelial cells by recombinant lymphotoxin: comparison with tumor necrosis factor and interleukin-1 species. J Immunol 138:3319–3324, 1987
40. Alexander MA, Benicelli J, Gurry D: Defective antigen presentation by human melanoma cell lines cultured from advanced, but not biologically, early disease. J Immunol 142:4070–4078, 1989
41. Kripke ML: Photoimmunology. Photochem Photobiol 52:919–924, 1990
42. Kripke ML: Immunology and photocarcinogenesis. J Am Acad Dermatol 14:149–156, 1986
43. Koh HK, Kligler BE, Lew RA: Sunlight and cutaneous malignant melanoma: evidence for and against causation. Photochem Photobiol 51:765–779, 1990
44. Köck A, Schwarz T, Kirnbauer R, et al: Human keratinocytes are a source for tumor necrosis factor-α: evidence for synthesis and release upon stimulation with endotoxin or ultraviolet light. J Exp Med 172:1609–1614, 1990